IONIC AND OSMOTIC CONCENTRATIONS IN BLOOD AND URINE OF PACHYGRAPSUS CRASSIPES ACCLIMATED TO DIFFERENT SALINITIES

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In its normal medium of sea water the blood of *Pachygrapsus* may be slightly hypo-osmotic (Jones, 1941; Robertson, 1953). In a dilute sea water the blood concentration declines somewhat but is maintained higher than the concentration of the medium (Jones, 1941) and this hyper-osmotic regulation permits the crab to enter regions of brackish water. In a more concentrated sea water *Pachygrapsus* shows limited hypo-osmotic regulation, a function associated with its survival out of water for long periods, as at low tide. Ionic analyses of blood as compared with sea water indicate that magnesium and sulfate are strongly excluded and that sodium, potassium and calcium concentrations are only slightly lower than in sea water (Robertson, 1953). If the crab is acclimated to sea water of various salinities, the ratio of potassium is held more constant than the sodium ratio (Gross, 1952).

The shore crabs' limited mechanisms of osmotic and ionic regulation are not well known but they are certainly interrelated. The excretory organs (antennary glands) appear to be more important for ionic than for osmotic regulation. In brackish water the osmotic concentration of the urine of *Carcinus maenas* is higher than the concentration of the medium and slightly lower than the concentration of the blood, yet the chloride in urine is the same as in blood; the volume of urine becomes increased but the combined effects of increased volume, slight dilution and no change in chloride concentration cannot account for the hyper-osmotic regulation (Nagel, 1934). A similar conclusion was reached on other grounds for *Palaemon* (Parry, 1954).

That various ions are excreted at separate rates is indicated by different blood/urine ratios for specific ions in such crabs as *Carcinus* (Robertson, 1949; Webb, 1940). Iodide which is injected into the crab is found to become concentrated in the urine (Nagel, 1934) and when extra $MgSO_4$ is added to sea water, magnesium and sulfate ions are increased in the urine (Webb, 1940) although urinary excretion accounts for loss of only a small fraction of injected salts (Bialasezwicz, 1932). To compensate for the high salt loss via urine when in a dilute medium, the crab may absorb salts actively, possibly by its gills (Webb, 1940); there may also be body salt stores which are used when the crab is in a dilute medium but these would necessarily be temporary (Hukada, 1932; Gross, 1951). The passive permeability of the body surface to both water and salts is low (Webb, 1940).

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Mechanisms of hypo-osmotic regulation in crabs are unknown, although active absorption of water is suggested (Gross, 1952). The mechanism of urine formation in the antennary glands is also unknown but filtration can hardly occur and secretion of salts and possibly of water is indicated (Prosser *et al.*, 1950).

A balance sheet of influx and outflux of water and ions by the various routes is needed. The following observations do not provide such a balance but they do partially indicate the role of the antennary glands in ionic and osmotic regulation in *Pachygrapsus crassipes*. *Pachygrapsus* is particularly suitable for investigation because it shows both hyper-osmotic and hypo-osmotic regulation, it can be catheterized easily for urine, it is of convenient size and survives well in the laboratory.

Materials and Methods

Freshly collected specimens of *Pachygrapsus crassipes* were acclimated to 50% and to 170% sea water (S.W.) over periods of five to seven days by changes of 10 to 30% per day; preliminary experiments showed virtually complete adaptation to such salinity changes in 24 hours. The crabs were kept in approximately 100 ml. of fluid in individual finger bowls at room temperature and the water was changed once or twice daily. Records were kept of sexes, but no differences noted; however, marked abnormalities in osmoregulation were found in molting crabs, as observed by Baumberger and Olmsted (1928); hence soft-shelled crabs were eliminated.

Blood samples were removed from sinuses by means of glass capillaries of 1 mm. O.D. and 2 cm. length by puncturing the cuticle at a leg joint. Urine was sampled by inserting the tips of capillaries, 0.1–0.3 mm. diameter, into the excretory pore; normally the pressure in the bladder caused the urine to overflow a 10 cm. long capillary at once. Drops of blood and urine were extruded from the capillaries onto a paraffin surface for sampling by micropipettes. Osmotic concentrations were measured in both blood and serum, ions in serum only.

Osmotic concentrations were measured by a freezing-point method similar to that developed by Gross (1952). Capillaries containing a small amount (0.01 ml. is sufficient) of fluid are sealed at the ends with vaseline, mounted in grooved plastic racks and quickly frozen on dry ice. A number of these racks of capillaries can be accumulated and on each rack are placed four or five capillaries containing known concentrations of NaCl; each rack holds 12 capillaries. For measurement, a rack of frozen capillaries is inserted into a holder which is immersed in a 300-ml. dish of brine (2 N NaCl) cooled by dry ice to lower than $-2°$ C. A stirrer agitates the brine which warms at about 1° per 15 minutes, a rate fixed by the amount of insulation in the box supporting the dish. Light from below passes through a sheet of polaroid, then through the dish with the capillaries, and through a plastic cover on which is a second piece of polaroid. When viewed by polarized light, the transition from the crystalline frozen state to the melted state is abrupt and sharp. The time of complete melting in each capillary is recorded, this time is plotted against the known concentrations, and values of unknowns are obtained by interpolation and expressed as equivalent normality of NaCl. Results of this method are easily reproducible to within 0.02 *N*; addition of 0.5% albumin to sea water causes no significant difference and values for serum and clotted blood overlap.

Sodium and potassium concentrations were measured with a Beckman flame photometer, using an acetylene flame. For the urine, 10-μl samples were read directly after dilution with Pyrex-distilled H_2O; blood samples were allowed to clot and serum was drawn into the micropipette. Sodium was read at 588 mμ and K at 767 mμ, against appropriate standards. Magnesium and calcium concentrations were measured by a Beckman flame photometer equipped with a hydrogen flame and a multiplier phototube similar to that described by Chow and Thompson (1955). Calcium and magnesium were read at 422.7 and 370.8 mμ, respectively.

Seven separate series of experiments were used with variations as indicated under Results. The total number of crabs examined after equilibration were: 31 in 100% S.W., 32 in 170% S.W., 20 in 50% S.W.

Results

In the first three series, osmo-concentration, potassium and sodium concentrations in blood (serum) and in urine were measured from groups of five or six crabs each in 50%, 100% and 170% S.W., respectively. In the fourth series some of the crabs from the second series were sampled a week later from 50%, 100% and 180% S.W.; these crabs were in poor condition, several deaths had occurred and it was concluded that experimental conditions were inadequate for such prolonged storage. In the fifth series of measurements, three groups of three crabs each were sampled at 1, 3 and 5 days after reaching 170% S.W. (this after 4 days of gradual transfer through intermediate concentrations); three groups of three crabs each in 100% S.W. sampled at the same time. No significant difference was found at the three times sampled; hence it was concluded that acclimation was virtually complete one day after reaching 170% S.W. Osmotic concentrations were measured on blood and serum in two-thirds of the animals, on blood in all. However, since differences between blood and serum values in a given medium do not differ by one standard error, only the blood figures are here presented.

The results of all the measurements of sodium, potassium and osmo-concentration in blood and urine of crabs acclimated to 50%, 100% and 170% S.W. are summarized in Table I and in Figure 1, b, c, and d and the significance in terms of 95 per cent fiducial limits for the blood are given in Table II. The osmotic concentration of the blood is clearly hypertonic to 50% S.W., it is insignificantly hypotonic in 100% S.W., and significantly hypotonic to 170% S.W. The urine is slightly hypotonic to the blood (10% level significance) in 50% S.W. and in 100% S.W. (3% significance). However in 170% S.W. the osmo-concentrations of blood and urine are virtually identical. It may be concluded that the antennary glands can play only a very minor part in hyper-osmotic regulation and no part at all in hypo-osmotic regulation.

The potassium concentration in blood from crabs in 100% S.W. is higher and in 50% S.W. is much higher than the potassium in the medium. The urine potassium concentration slightly exceeds that in the blood in crabs from 50% S.W. but this difference is not significant (10% level) and there is no difference in 100% S.W. and 170% S.W. It appears, therefore, that the antennary glands do not

function in regulation of potassium but rather that urine potassium reflects blood levels.

Sodium in blood is more concentrated than in the medium when the animal is in 50% S.W. but the difference is less than for osmo-concentration; in other concentrations of sea water the blood sodium does not differ significantly from the medium. Hence osmotic regulation is determined mainly by other than

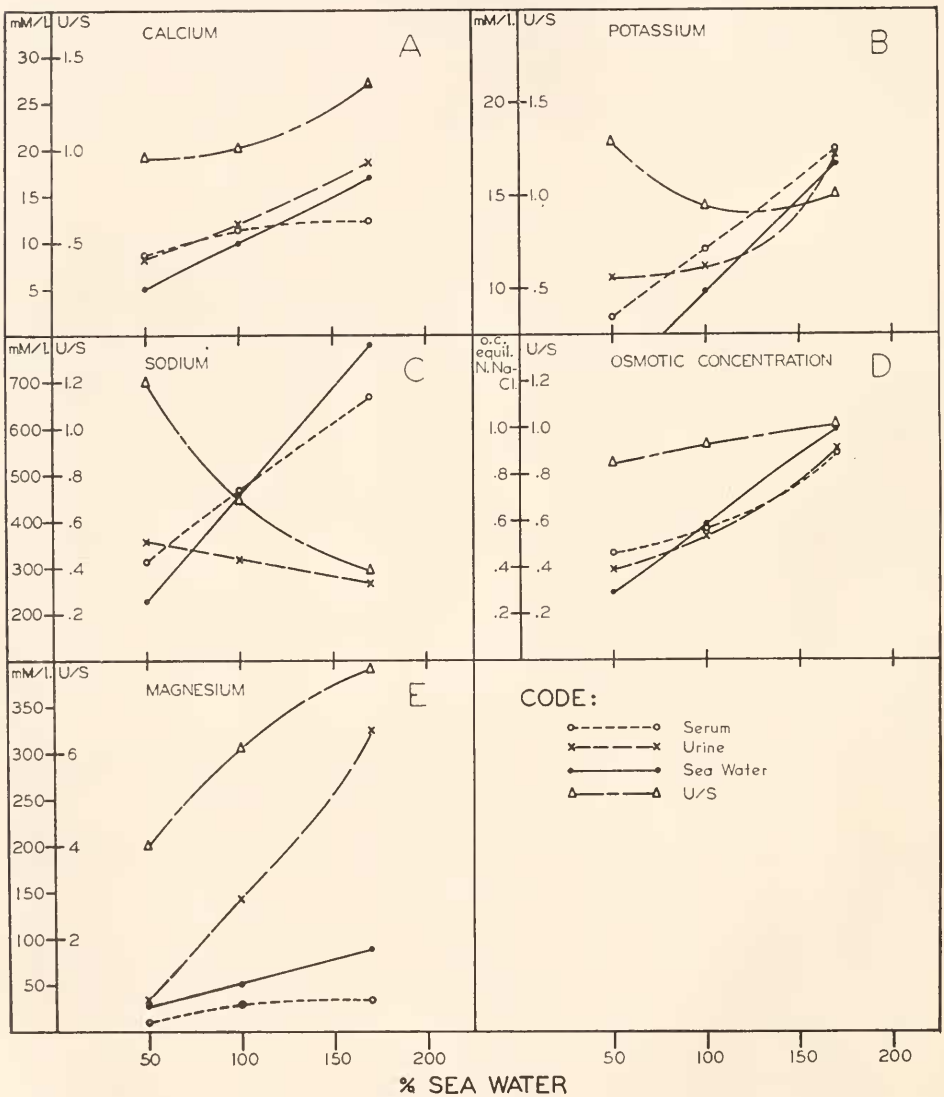

FIGURE 1. Average data for concentrations of calcium, potassium, sodium, serum, urine and external medium in m mols per liter and for osmotic concentration in equivalent normality of NaCl. U/S gives urine/serum ratios. Number of crabs tested in each concentration of sea water is given in Tables I and IV.

TABLE I

Sodium, potassium, and osmotic concentrations in blood and urine of crabs from 50%, 100%, and 170% S.W.

	50% S.W.			100% S.W.			170% S.W.		
	Avg.	S.E.	No. crabs	Avg.	S.E.	No. crabs	Avg.	S.E.	No. crabs
Sodium									
(mM/1) Serum	313	±12.0	18	465	±11.2	30	668	±12.0	31
(mM/1) Urine	356	±17.3	17	318	±16.7	27	264	±19.2	29
U/S ratios	1.2	± 0.1	17	0.7	± 0.04	26	0.39	± 0.024	29
Potassium									
(mM/1) Serum	8.4	± 0.78	13	12.1	± 0.65	30	17.4	± 0.66	31
(mM/1) Urine	10.6	± 0.94	13	11.2	± 0.59	28	17.2	± 0.77	31
U/S ratios	1.29	± 0.09	13	0.94	± 0.05	27	1.00	± 0.04	31
Osmotic concentration									
(equiv. N NaCl) Blood	0.46	± 0.026	18	0.57	± 0.013	24	0.89	± 0.023	30
(equiv. N NaCl) Urine	0.39	± 0.031	19	0.53	± 0.013	25	0.90	± 0.02	29
U/B ratios	0.85	± 0.04	18	0.93	± 0.03	24	1.00	± 0.01	29

sodium ions. However the sodium concentration in urine is higher than in serum (significant only at 5% level) in 50% S.W., is lower in urine than in blood when in 100% S.W. and much lower when in 170% S.W. Thus the sodium concentration in urine *decreases*, while in blood it *increases* with increasing environmental concentration.

TABLE II

Statistical significance of analyses

A. Test of concentrations in blood as compared with medium.

	50% S.W.		100% S.W.		170% S.W.	
	Blood 95% limits	S.W.	Blood 95% limits	S.W.	Blood 95% limits	S.W.
Osmotic conc. (equiv. N NaCl)	0.41–0.51	.29	.54–.60	.58	.84–.94	.99
Na (mM/1)	288–338	229	435–495	459	639–697	780
K (mM/1)	6.7–10.1	4.9	10.8–13.4	9.8	16.1–18.7	16.7

B. Test of blood and urine differences.

	50% S.W.		100% S.W.		170% S.W.	
	t	Significance	t	Significance	t	Significance
Osmotic conc.	1.68	$P < 0.1$	2.17	$P \sim .03$	.33	not sig.
Na	2.05	$P = .05$	7.3	$P < .01$	1.75	$P < .01$
K	1.80	$P > 0.1$	1.02	not sig.	.19	not sig.

TABLE III

Averages of analyses of blood and urine from crabs in artificial sea water (average of 3 crabs each)

	Na			K			Osmotic conc.		
	Serum	Urine	U/S	Serum	Urine	U/S	Blood	Urine	U/B
A. From normal artificial sea water.									
100% S.W.	455	319	.79	12.2	7.5	.61	.59	.60	1.06
170%	653	200	.31	14.0	9.8	.77	.78	.75	.96
B. From $MgSO_4$-free artificial S.W.									
100%	422	463	1.09	10.1	12.3	.95	.52	.53	1.01
170%	686	514	.75	13.5	15.1	1.11	.92	.93	1.01

Since urinary sodium decreases while urine osmo-concentration and blood osmo- and sodium concentrations increase, some other important solute must be replacing the sodium in the urine. Magnesium is the next most abundant cation in sea water. To test its possible role an experiment was conducted with crabs in artificial sea water, 100% and 170%, made up with and without $MgSO_4$. Sodium chloride was added to compensate osmotically in the $MgSO_4$-free sea water. Three crabs were acclimated in each concentration of each mixture. Results as given in Table III indicate that the sodium concentration in the urine decreased at the change from 100% to 170% artificial sea water as in normal sea water, but that in the absence of $MgSO_4$ the urine Na increased when the tonicity of the medium was increased. Also the urine sodium was higher in the $MgSO_4$-free 100% artificial S.W. than in the 100% normal S.W. It is concluded that the urine/serum ratio for sodium is reduced when Mg and SO_4 are present; that is, magnesium seems to suppress the urinary excretion of sodium.

In two series of experiments determinations of magnesium and calcium were also made. The data for Na, K and osmotic concentration in these animals have

TABLE IV

Summary of Mg and Ca analyses

		50% S.W.		100% S.W.		170% S.W.	
		mM/l S.E.	No.	mM/l S.E.	No.	mM/l S.E.	No.
Mg	serum	8.9 ±.73	3	29.2 ±.93	4	33.1 ±.59	4
	urine	32.1	2	143.6 ±21.2	5	324.6 ±.43	6
	U/S	4.0	2	6.1 ±.86	3	7.8 ±.47	4
	Sea water	26.0		52.0		88.5	
Ca	serum	8.6 ±.5	3	11.4 ±.60	4	12.3 ±1.1	4
	urine	8.4 ±.34	3	12.0 ±.56	6	18.6 ±2.2	6
	U/S	0.97±.02	3	1.01±.04	4	1.36±0.2	4
	Sea water	5.0		10.0		17.0	

been included in Table I, and the data for Mg and Ca are summarized in Table IV and Figure 1, a and e. The calcium level in both serum and urine increased as the total concentration of the medium increased. In fact, calcium appears to follow the pattern of potassium.

Serum magnesium increased proportionately more than any other constituent measured, nearly four-fold in going from 50% to 170% S.W. and the urine magnesium increased by nearly eight times. Thus as urine sodium decreases, urine magnesium increases.

Discussion

The preceding results confirm previous investigations in indicating differences in degree of regulation of different blood components. For a 50% dilution of sea water the per cent change in the measured components of blood in order of decreasing regulation is: osmotic concentration 19, calcium 25, potassium 31, sodium 33, magnesium 70. For a 70% increase in concentration of the medium the per cent change, also in order of decreasing regulation, is: calcium 7.9, magnesium 13, potassium 44, sodium 44 and osmotic concentration 56. The values for Mg and Ca are based on fewer measurements than the others but it is evident that each component is regulated to a different degree and in a different order for dilution than for concentration. Hyper-osmotic regulation is not the converse of hypo-osmotic regulation. These observations, in general, agree with and extend those of Jones (1941), Robertson (1953) and Gross (1952).

The role of the antennary glands is indicated by urine/serum ratios in Tables I and IV. Deviation of the U/S ratio from unity is a measure of effectiveness of the antennary glands in regulation. In 100% S.W. this deviation is significant to better than the 1% level for sodium and magnesium; it is of borderline significance for potassium and osmotic concentration and insignificant for calcium. In 50% S.W. the U/S ratio deviates from unity significantly for magnesium, sodium and potassium, questionably for osmotic concentration. In 170% S.W. only sodium and magnesium show U/S ratios significantly different from unity. It may be concluded that the antennary glands function in eliminating magnesium at all levels and that they tend to retain sodium and potassium in a dilute medium. In 170% S.W. the sodium concentration is similar in the medium, in the serum and in the urine, hence no active excretion might be demanded. However, since the blood is hypotonic to the medium and urinary magnesium is greatly increased, some influx of salts must occur and extra-renal excretion of sodium is likely. In hypo-osmotic marine teleosts magnesium and calcium are excreted renally, sodium and potassium extra-renally (Smith, 1930), whereas in *Pachygrapsus* urinary potassium increases along with magnesium and thus separates from the route taken by sodium.

Most unexpected is the decrease in urine sodium as the total urine concentration increases. The experiments with artificial sea water and the direct analyses indicate that the preferential route of Mg excretion is renal and that excretion of Mg interferes with the excretion of Na by the antennary glands. Thus *Pachygrapsus* differs from *Leander* (Parry, 1954) where urine magnesium increases more with total concentration than sodium but the sodium does not decrease. It would be of interest to learn whether the inverse re-

lation of sodium and magnesium results from competitive interference in an enzyme system or from some other type of blocking of the sodium transport system by magnesium. Calculations indicate that magnesium salts are not totally adequate to account for the osmotic deficit in the urine as sodium decreases. For example, if it be assumed that each of the cations is excreted with chloride, the isotonic coefficients in the range of urine concentrations are 1.82 for NaCl, 1.9 for KCl and 2.6 for the divalent salts (from Internat. Crit. Tables). The computed total milliosmolar concentrations due to salts in urine are in 50% S.W. 766 compared with 704 measured, in 100% S.W. 997 compared with 955 measured, and in 170% S.W. 1398 compared with 1615 measured. If it is assumed that magnesium and calcium are excreted with sulphate, the isotonic coefficients of these salts are 1.2 and the calculated total osmotic concentrations due to the salts in the urine are 719, 789 and 927 milliosmoles in the three sea water concentrations. Hence some unknown solute must be present in quantity in the concentrated urines. In any case, complex secretory activity, both renal and extra-renal, is indicated for osmotic and ionic regulation in *Pachygrapsus*.

Summary

1. When specimens of *Pachygrapsus crassipes* were acclimated to 50%, 100% and 170% sea water the average blood osmotic concentrations were equivalent to 0.46, 0.57 and 0.89 normal NaCl, as compared with the medium of 0.29, 0.58 and 0.99, respectively. Urine osmotic concentrations in the same series were equivalent to 0.39, 0.53 and 0.90 normal NaCl.

2. Thus the crabs are hyper-osmotic in a dilute medium and hypo-osmotic in a concentrated medium; the antennary glands may function slightly in hyper-osmotic regulation but not at all in hypo-osmotic regulation.

3. Serum sodium concentrations in 50, 100 and 170% S.W. were 313, 465 and 668 mM, urine sodium 356, 318 and 264 mM corresponding to environmental concentrations of 229, 459 and 780 mM. Thus as the blood sodium increases the urine sodium decreases.

4. The osmotic deficit in the urine is accounted for in part by magnesium which in 50, 100 and 170% S.W. was in blood 9, 29 and 33 mM, in urine 32, 144 and 345 mM while the medium was 26, 52 and 88 mM, respectively.

5. In artificial sea water urinary sodium decreased at high external salinity but in the absence of $MgSO_4$, the urinary sodium increased.

6. Active outward transport of magnesium by the antennary glands in some way reduces the excretion of sodium.

7. Potassium in blood is well regulated in dilute medium, less well in more concentrated sea water. Calcium in blood is more concentrated than in the dilute medium, less concentrated than in the higher salinity medium.

LITERATURE CITED

Baumberger, J. P., and J. M. D. Olmsted, 1928. Changes in the osmotic pressure and water content of crabs during the molt cycle. *Physiol. Zool.*, **1**: 531–544.

Bialaszewicz, K., 1932. Sur la regulation de la composition minérale de l'hémolymphe chez le crabe. *Arch. Int. Physiol.*, **35**: 98–124.

Chow, T. J., and T. G. Thompson, 1955. Flame photometric determinations of strontium in sea water. *Anal. Chem.*, **27**: 18–21.

Gross, W. J., 1952. Studies of response to osmotic stress in selected invertebrates. Ph.D. dissertation, University of California at Los Angeles.

Hukada, K., 1932. Changes of weight of marine animals in diluted media. *J. Exp. Biol.*, **9**: 61–68.

Jones, L. L., 1941. Osmotic regulation in several crabs of the Pacific coast of North America. *J. Cell. Comp. Physiol.*, **18**: 79–92.

Nagel, H., 1934. Die Aufgaben der Exkretionsorgane und der Kiemen bei der Osmoregulation von *Carcinus maenas*. *Zeit. vergl. Physiol.*, **21**: 468–491.

Parry, G., 1954. Ionic regulation in the palaemonid prawn, *Palaemon* (= *Leander*) *serratus*. *J. Exp. Biol.*, **31**: 601–613.

Prosser, C. L., F. A. Brown, D. Bishop, T. L. Jahn and V. J. Wulff, 1950. Ch. II, pp. 6–74 in Comparative Animal Physiology. W. B. Saunders Co., Phila.

Robertson, J. D., 1949. Ionic regulation in some marine invertebrates. *J. Exp. Biol.*, **26**: 182–200.

Robertson, J. D., 1953. Further studies on ionic regulation in marine invertebrates. *J. Exp. Biol.*, **30**: 277–296.

Smith, H. W., 1930. The absorption and excretion of water and salts by marine teleosts. *Amer. J. Physiol.*, **93**: 480–505.

Webb, D. A., 1940. Ionic regulation in *Carcinus maenas*. *Proc. Roy. Soc. Lond., Ser B*, **129**: 107–135.